

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1. (Original) A method of producing (S)-2-pentanol which comprises allowing microorganisms or transformed cells, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-pentanone, wherein when a fresh cell mass of said microorganisms or transformed cells, which has not been pretreated with a solvent, is allowed to act on 2-pentanone, (S)-2-pentanol having an optical purity of 95% e.e. or greater can be generated, and the productivity thereof is 1 mg or more of (S)-2-pentanol/g of dry cell mass weight/hour.

2. (Original) A method of producing (S)-2-hexanol which comprises allowing microorganisms or transformed cells, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or

cells, to act on 2-hexanone, wherein when a fresh cell mass of said microorganisms or transformed cells, which has not been pretreated with a solvent, is allowed to act on 2-hexanone, (S)-2-hexanol having an optical purity of 95% e.e. or greater can be generated, and the productivity thereof is 1 mg or more of (S)-2-hexanol/g of dry cell mass weight/hour.

3. (Original) A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein microorganisms selected from the group consisting of genus *Brettanomyces*, genus *Candida*, genus *Hortaea*, genus *Issatchenkia*, genus *Lodderomyces*, genus *Pichia*, genus *Rhodotorula*, genus *Arthrobacter*, genus *Brevibacterium*, genus *Crutobacterium*, genus *Geobacillus*, genus *Microbacterium*, genus *Ochrobactrum*, genus *Paracoccus*, genus *Rhizobium*, and genus *Rhodococcus*, a product obtained by treating said microorganisms, a culture solution of said microorganisms, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol.

4. (Original) A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein transformed cells wherein DNA encoding carbonyl reductase obtained from microorganisms selected from

the group consisting of genus *Brettanomyces*, genus *Candida*, genus *Hortaea*, genus *Issatchenkia*, genus *Lodderomyces*, genus *Pichia*, genus *Rhodotorula*, genus *Arthrobacter*, genus *Brevibacterium*, genus *Curtobacterium*, genus *Geobacillus*, genus *Microbacterium*, genus *Ochrobactrum*, genus *Paracoccus*, genus *Rhizobium*, and genus *Rhodococcus*, has been allowed to express, a product obtained by treating said cells, a culture solution of said cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said cells, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol.

5. (Currently Amended) The production method according to claim 3 or —4—, wherein the microorganisms are selected from the group consisting of *Brettanomyces bruxellensis*, *Brettanomyces anomalus*, *Candida famata*, *Candida krusei*, *Candida maltosa*, *Candida tropicalis*, *Candida zeylanoides*, *Hortaea werneckii*, *Issatchenkia scutulata*, *Lodderomyces elongisporus*, *Pichia angusta*, *Pichia besseyi*, *Pichia cactophila*, *Pichia segobiensis*, *Pichia spartinae*, *Pichia trehalophila*, *Rhodotorula minuta*, *Arthrobacter oxydans*, *Arthrobacter polychromogenes*, *Arthrobacter* sp., *Arthrobacter sulfurous*, *Brevibacterium butanicum*, *Curtobacterium flaccumfaciens*, *Geobacillus stearothermophilus*, *Microbacterium keratanolyticum*, *Microbacterium saperdae*, *Microbacterium* sp., *Microbacterium testaceum*,

*Ochrobactrum anthropi*, *Ochrobactrum* sp. (*Pseudomonas ovalis*),  
*Pracoccus denitrificans*, *Rhizobium radiobacter*, and *Rhodococcus* sp.  
(*Corynebacterium hydrocarboclastum*).

6. (Original) A method for producing (S)-2-pentanol or (S)-2-hexanol having high optical purity, wherein transformed cells, wherein DNA described in any one of the following (A) to (F) has been allowed to express, a product obtained by treating said cells, and/or a culture solution of said cells, are allowed to act on 2-pentanone or 2-hexanone, so as to generate (S)-2-pentanol or (S)-2-hexanol:

(A) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1;

(B) DNA encoding a protein, which has an amino acid sequence comprising a deletion, addition, or substitution of one or several amino acids with respect to the amino acid sequence shown in SEQ ID NO: 1, and which has ability to reduce a carbonyl group to synthesize optically active alcohol;

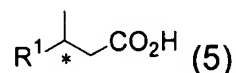
(C) DNA encoding a protein, which has an amino acid sequence showing homology of 50% or more with the amino acid sequence shown in SEQ ID NO: 1, and which has ability to reduce a carbonyl group to synthesize optically active alcohol;

(D) DNA having the nucleotide sequence shown in SEQ ID NO: 2;

(E) DNA having a nucleotide sequence, which comprises a deletion, addition, or substitution of one or several nucleotides with respect to the nucleotide sequence shown in SEQ ID NO: 2, and which encodes a protein having ability to reduce a carbonyl group to synthesize optically active alcohol; and

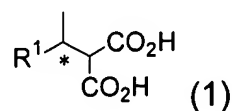
(F) DNA having a nucleotide sequence, which hybridizes with the nucleotide sequence shown in SEQ ID NO: 2 or a complementary sequence thereof under stringent conditions, and which encodes a protein having ability to reduce a carbonyl group to synthesize optically active alcohol.

7. (Original) A method for producing (R)- or (S)-3-methyl carboxylic acid represented by the following formula (5):



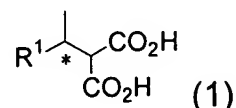
wherein R<sup>1</sup> represents an alkyl group containing 3 to 5 carbon atoms, and \* represents an asymmetric carbon,

which comprises decarboxylating (R)- or (S)-1-methylalkyl malonic acid having optical activity represented by the following formula (1) in the presence of a highly polar solvent and/or an additive for promoting decarboxylation:



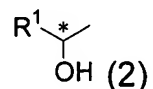
wherein R<sup>1</sup> has the same definition as described above, and \* represents an asymmetric carbon.

8. (Original) A method for producing (R)- or (S)-1-methylalkyl malonic acid represented by the following formula (1):

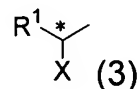


wherein R<sup>1</sup> represents an alkyl group containing 3 to 5 carbon atoms, and \* represents an asymmetric carbon,

which comprises allowing optically active alcohol represented by the following formula (2) to react with a sulfonylation agent:

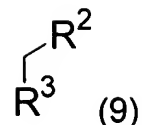


wherein R<sup>1</sup> has the same definition as described above, and \* represents an asymmetric carbon, so as to obtain an optically active compound represented by the following formula (3):

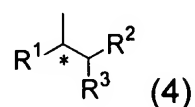


wherein R<sup>1</sup> has the same definition as described above, X represents a sulfonyloxy group, and \* represents an asymmetric carbon;

allowing the optically active compound to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:



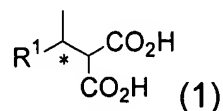
wherein each of  $\text{R}^2$  and  $\text{R}^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $\text{R}^2$  and  $\text{R}^3$  may together form a cyclic structure, so as to obtain an optically active compound represented by the following formula (4):



wherein  $\text{R}^1$ ,  $\text{R}^2$ , and  $\text{R}^3$  have the same definitions as described above, and \* represents an asymmetric carbon, and

hydrolyzing the obtained optically active compound.

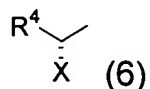
9. (Original) (R)-1-methylalkyl malonic acid or (S)-1-methylalkyl malonic acid having an optical purity of 90%ee or greater, which is represented by the following formula (1):



wherein  $\text{R}^1$  represents an alkyl group containing 3 to 5 carbon atoms, and \* represents an asymmetric carbon.

10. (Original) The (R)-1-methylalkyl malonic acid or (S)-1-methylalkyl malonic acid according to claim 9, wherein  $\text{R}^1$  represents an n-propyl group or an n-butyl group.

11. (Original) A method for producing an optically active substance represented by the following formula (6):

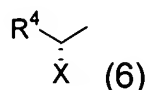


wherein R<sup>4</sup> represents an n-propyl group, and X represents a sulfonyloxy group,

which comprises: allowing microorganisms or transformed cells containing a carbonyl reductase having activity to react with 2-pentanone to generate (S)-2-pentanol, wherein it is able to generate (S)-2-pentanol having an optical purity of 95% e.e. or greater when the fresh cell mass thereof, which has not been pretreated with a solvent, is allowed to act on 2-pentanone, and the productivity thereof is 10 mg or more of (S)-2-pentanol/g of dry cell mass weight/hour, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-pentanone, so as to convert it to (S)-2-pentanol; and allowing the obtained (S)-2-pentanol to react with a sulfonylation agent, so as to convert it to the optically active substance represented by the above formula (6).



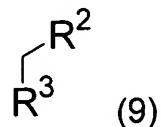
12. A method for producing an optically active substance represented by the following formula (6):



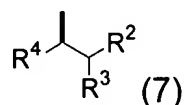
wherein R<sup>4</sup> represents an n-butyl group, and X represents a sulfonyloxy group,

which comprises: allowing microorganisms or transformed cells containing a carbonyl reductase having activity to react with 2-hexanone to generate (S)-2-hexanol, wherein it is able to generate (S)-2-hexanol having an optical purity of 95% e.e. or greater when the fresh cell mass thereof, which has not been pretreated with a solvent, is allowed to act on 2-hexanone, and the productivity thereof is 10 mg or more of (S)-2-hexanol/g of dry cell mass weight/hour, a product obtained by treating said microorganisms or cells, a culture solution of said microorganisms or cells, and/or a crude purified product or purified product of a carbonyl reductase fraction obtained from said microorganisms or cells, to act on 2-hexanone, so as to convert it to (S)-2-hexanol; and allowing the obtained (S)-2-hexanol to react with a sulfonylation agent, so as to convert it to the optically active substance represented by the above formula (6).

13. (Currently Amended) The method according to claim 11 or 12, which further comprises a step of allowing the optically active substance represented by formula (6) to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

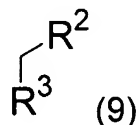


wherein each of  $\text{R}^2$  and  $\text{R}^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $\text{R}^2$  and  $\text{R}^3$  may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):

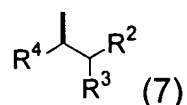


wherein  $\text{R}^2$  and  $\text{R}^3$  have the same definitions as described above, and  $\text{R}^4$  represents an n-propyl group or an n-butyl group.

14. (Currently Amended) A method for producing (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid, which comprises; allowing the optically active substance represented by formula (6) obtained by the method according to claim 11 or 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

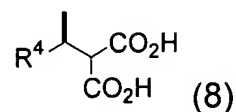


wherein each of  $R^2$  and  $R^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $R^2$  and  $R^3$  may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



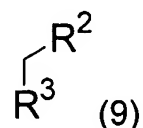
wherein  $R^2$  and  $R^3$  have the same definitions as described above, and  $R^4$  represents an n-propyl group or an n-butyl group, and

hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):

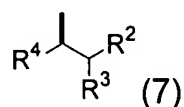


wherein  $R^4$  has the same definition as described above.

15. (Currently Amended) A method for producing (R)-3-methyl hexanoic acid or (R)-3-methyl heptanoic acid, which comprises allowing the optically active substance represented by formula (6) obtained by the method according to claim 11 or 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

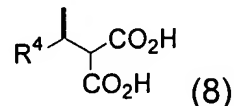


wherein each of  $R^2$  and  $R^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $R^2$  and  $R^3$  may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



wherein  $R^2$  and  $R^3$  have the same definitions as described above, and  $R^4$  represents an n-propyl group or an n-butyl group,

hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):



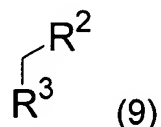
wherein  $R^4$  has the same definition as described above, and

decarboxylating the obtained (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid.

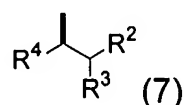
16. (New) The production method according to claim 4, wherein the microorganisms are selected from the group consisting of *Brettanomyces bruxellensis*, *Brettanomyces anomalus*, *Candida famata*, *Candida krusei*, *Candida maltosa*, *Candida tropicalis*, *Candida zeylanoides*, *Hortaea werneckii*, *Issatchenkia scutulata*, *Lodderomyces elongisporus*, *Pichia angusta*, *Pichia besseyi*, *Pichia cactophila*, *Pichia segobiensis*, *Pichia*

*spartinae*, *Pichia trehalophila*, *Rhodotorula minuta*, *Arthrobacter oxydans*, *Arthrobacter polychromogenes*, *Arthrobacter* sp., *Arthrobacter sulfurous*, *Brevibacterium butanicum*, *Curtobacterium flaccumfaciens*, *Geobacillus stearothermophilus*, *Microbacterium keratanolyticum*, *Microbacterium saperdae*, *Microbacterium* sp., *Microbacterium testaceum*, *Ochrobactrum anthropi*, *Ochrobactrum* sp. (*Pseudomonas ovalis*), *Pracoccus denitrificans*, *Rhizobium radiobacter*, and *Rhodococcus* sp. (*Corynebacterium hydrocarboclastum*).

17. (New) The method according to claim 12, which further comprises a step of allowing the optically active substance represented by formula (6) to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

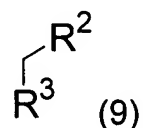


wherein each of  $\text{R}^2$  and  $\text{R}^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $\text{R}^2$  and  $\text{R}^3$  may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):

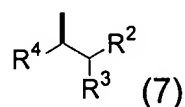


wherein  $R^2$  and  $R^3$  have the same definitions as described above, and  $R^4$  represents an n-propyl group or an n-butyl group.

18. (New) A method for producing (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid, which comprises; allowing the optically active substance represented by formula (6) obtained by the method according to claim 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

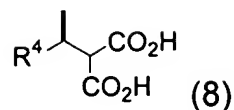


wherein each of  $R^2$  and  $R^3$  independently represents an ester group, a carboxyl group, or a cyano group, wherein  $R^2$  and  $R^3$  may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



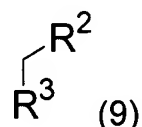
wherein  $R^2$  and  $R^3$  have the same definitions as described above, and  $R^4$  represents an n-propyl group or an n-butyl group, and

hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):

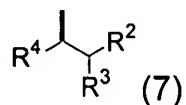


wherein R<sup>4</sup> has the same definition as described above.

19. (New) A method for producing (R)-3-methyl hexanoic acid or (R)-3-methyl heptanoic acid, which comprises allowing the optically active substance represented by formula (6) obtained by the method according to claim 12 to react with a carbon nucleophile represented by the following formula (9) in the presence of a base:

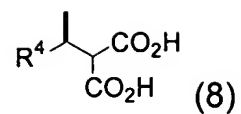


wherein each of R<sup>2</sup> and R<sup>3</sup> independently represents an ester group, a carboxyl group, or a cyano group, wherein R<sup>2</sup> and R<sup>3</sup> may together form a cyclic structure, so as to convert it to an optically active compound represented by the following formula (7):



wherein R<sup>2</sup> and R<sup>3</sup> have the same definitions as described above, and R<sup>4</sup> represents an n-propyl group or an n-butyl group,

hydrolyzing the obtained optically active compound, so as to convert it to (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid represented by the following formula (8):



wherein R<sup>4</sup> has the same definition as described above, and

decarboxylating the obtained (R)-1-methylbutyl malonic acid or (R)-1-methylpentyl malonic acid.